Molecular and cellular effects of the Ogden syndrome S37P mutation on the function of the N-terminal acetyltransferase Naa10

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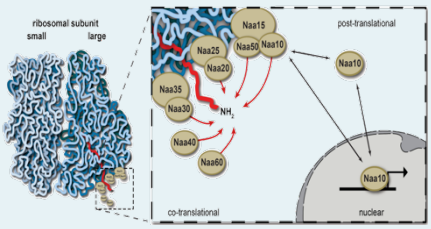
Background

N$^{\alpha}$-terminal acetylation is one of the most common protein modifications in eukaryotes. Specific N$^{\alpha}$-terminal acetyltransferases (NATs) catalyze the transfer of an acetyl group from acetyl-CoA to the very N-terminal amino group of their corresponding substrates. In humans, six NATs (NatA-NatF) with specific substrate specificity have been identified [1].

NatA is composed of an auxiliary subunit, Naa15, and the catalytic subunit, Naa10. Naa15 links Naa10 and Naa50, the catalytic subunit of NatE, to the ribosome. Naa10 co-translationally acetylates proteins starting with small side chains such as Ser, Ala, Gly, Thr or Cys after the initiator methionine has been cleaved by ubiquitous methionine aminopeptidases [2].

Recently, we have identified a S37P mutation in the NAA10 gene as contributing to a lethal disease of infancy that we named Ogden syndrome [3]. Ogden syndrome is characterized by a distinct combination of an aged appearance, craniofacial anomalies, hypotonia, global developmental delays, cryptorchidism and cardiac arrhythmias.



Cellular function of Naa10: Associated with the ribosome in the NatA complex, Naa10 co-translationally acetylates the N$^{\alpha}$-terminal amino group of the nascent polypeptide chains of classical substrates as they emerge from the ribosome. Uncomplexed Naa10 post-translationally N$^{\alpha}$-acetylates proteins starting with acidic side chains and might also N$^{\varepsilon}$-acetylate internal lysines. Furthermore, Naa10 translocates into the nucleus where it acts in cooperation with transcription factors to modulate protein expression.

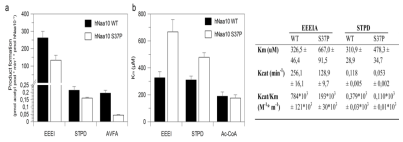
Methods

Here we use HEK293 cells and primary fibroblasts to study the effects of a single mutation (S37P) in the human N$^{\alpha}$-acetyltranferase Naa10.

The methods include *in vitro*-acetylation assays, immuno-fluorescence staining and co-immunorecipitation assays combined with quantitative mass spectrometry (isobaric iTRAQ labeling and 2D MudPIT LCMS prior to analysis using a Thermo Velos Orbitrap) to study the underlying effects of the Ogden S37P mutation.

Acetyltransferase activity is reduced in the S37P mutation

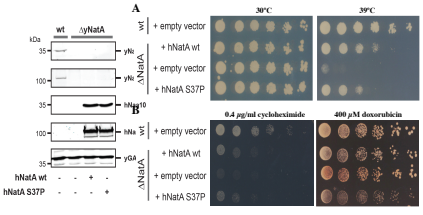
Affinity purified recombinant MBP-hNaa10 WT and MBP-hNaa10 S37P was incubated with oligopeptides (EEEI, STPD and AVFA) and Acetyl -CoA . **(A)** Product formation was quantified by RP-HPLC. **(B)** Varying concentrations of either peptide or Acetyl-CoA was used to calculate the Km and Vmax values.



	EEEIA		STPD	
	WT	S37P	WT	S37P
Km (uM)	326,5 ± 46,4	667,0 ± 91,1	310,9 ± 28,9	478,3 ± 34,7
Kcat (min^{-1})	256,1 ± 16,1	128,9 ± 9,7	0,118 ± 0,005	0,053 ± 0,002
Kcat/Km ($M^{-1}*m^{-1}$)	784*10^3 ± 12*10^3	193*10^3 ± 30*10^3	0,379*10^3 ± 0,03*10^3	0,110*10^3 ± 0,8*10^3

Yeast ΔNatA cells display growth defects under stress conditions

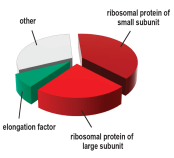
yNatA knockout strain and rescue strains with plasmids expressing hNaa15/hNaa10 wt or S37P mutant to analyze the effects of the Ogden mutation under stress conditions. **A)** Serial dilutions were spotted on SD plates and grown at 30ºC or 39ºC. **B)** Serial dilutions were spotted on SD plates +/-cycloheximide or doxorubicin.



S37P mutation de-regulates ribosomal proteins

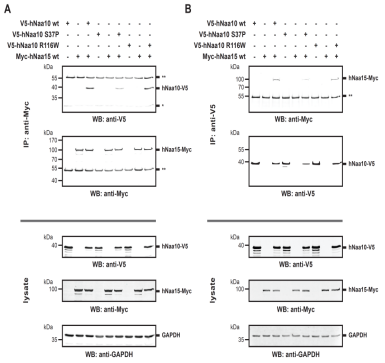
Yeast cultures were lysed and proteins and chemically labeled separately with 1 of 4 distinct isobaric iTRAQ reagents and subjected to a standard 2D MudPIT LCMS and analyzed using a Thermo Velos Orbitrap mass spectrometer. 2130 proteins were identified in total and screened for specific changes in yNatA

SGD Database	Description	k/o	hNaa10 wt	hNaa10 S37P
S000004751	Ribosomal protein S16A	-0.83	0.04	-0.41
S000004843	Ribosomal protein 10B	-0.75	0.07	-0.34
S000002858	Ribosomal protein S19A	-0.74	0.07	-0.36
S000000393	Ribosomal protein 9B	-0.58	-0.03	-0.44
S000002969	ERG26	-0.55	0.08	-0.37
S000003221	RTG2	-0.54	0.06	-0.51
S000003726	Ribosomal protein S22A	-0.51	-0.03	-0.50
S000004854	YHM2	-0.48	0.00	-0.40
S000000743	Glutathione Transferase 3	-0.45	0.09	-0.37
S000001395	Ribosomal protein L16B	-0.44	0.16	-0.37
S000000436	Ribosomal protein L22A	-0.44	0.24	-0.20
S000001395	Ribosomal protein L16A	-0.43	0.16	-0.39
S000001643	Elongation factor 1	-0.61	0.09	-0.52



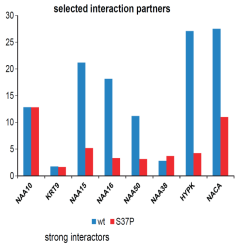
The S37P mutation diminishes NatA complex formation

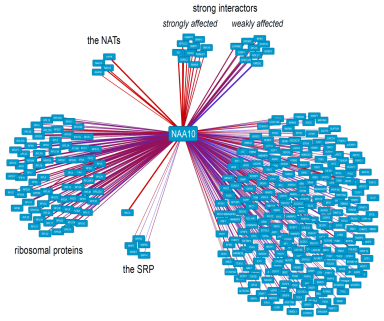
HEK293 were transiently transfected with empty vector, V5-Naa10 wt or V5-Naa10 S37P, respectively. Protein complexes were isolated using Myc- **(A)** or V5-antibody **(B)** and analyzed on SDS-PAGE.



hNaa10 is associated with the ribosome and the NAC complex

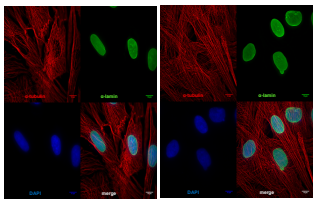
Co-immunoprecipitation as above. Bound proteins were chemically labeled with 1 of 3 isobaric iTRAQ reagents analyzed on a Thermo Velos Orbitrap mass iTRAQ. The relative abundance of the peptides were deduced directly from the relative intensities of the corresponding reporter ions.





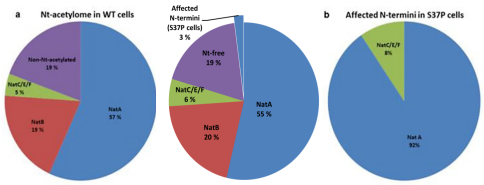
Primary fibroblasts from patient with Ogden syndrome

The symptoms of the Ogden boys suggest an overlap between Hutchinson-Gilford progeria syndrome (HGPS), but immunofluorescence staining of lamin A+C from patient primary fibroblast show no defects of the nuclear lamina, a main criteria of HGPS.



Effect of the Naa10-S37P mutation in fibroblasts on N$^{\alpha}$-terminal acetylation

N-terminal combined fractional diagonal chromatography (COFRADIC) and mass spectrometric analyses of the N-acetylome of B-cells derived from the Ogden patient and an unaffected brother. **(A)** N-terminal sequences in the proteome of WT (left) and S37P cells (right). 1066 unique N-termini were identified in both setups. **(B)** represents peptides with >10% Nt-acetylation shift in S37P cells as compared to WT (32 unique N-termini).



Conclusion

The results presented here, indicate that the S37P mutation in Naa10 decreases the catalytic acetyltransferase activity of Naa10 *in vitro* and *in vivo* and disrupts complex formation with its auxiliary subunit and ribosomal proteins. The yeast model revealed a growth defect and de-regulation of ribosomal proteins. Therefore, we speculate that the Ogden mutation may lead to a translational defect, that could explain the severity of this disease.

References

[1] T. V. Kalvik and T. Arnesen, "Protein N-terminal acetyltransferases in cancer," *Oncogene*, vol. 32, pp. 269-76, Jan 2013.

[2] P. Van Damme, K. Hole, A. Pimenta-Marques, K. Helsens, J. Vandekerckhove, R. G. Martinho, *et al.*, "NatF contributes to an evolutionary shift in protein N-terminal acetylation and is important for normal chromosome segregation," *PLoS Genet*, vol. 7, p. e1002169, Jul 2011.

[3] A. F. Rope, K. Wang, R. Evjenth, J. Xing, J. J. Johnston, J. J. Swensen, *et al.*, "Using VAAST to identify an X-linked disorder resulting in lethality in male infants due to N- terminal acetyltransferase deficiency," *Am J Hum Genet*, vol. 89, pp. 28-43, Jul 2011.